stability and ability to enter cells without lipid formulation, that they may find utility as a sponge for disease-related miRNAs. Given the noted tolerance of the liver to high doses of the ss-siRNAs, they could conceivably be used to inhibit miRNAs involved in disease pathogenesis. Indeed, tiny locked nucleic acids (LNAs) with complementarity to miRNAs show promise in reducing miRNA activity (Obad et al., 2011).

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Holding on through DNA Replication: Histone Modification or Modifier?

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Histone methylation is widely believed to contribute to epigenetic inheritance by persevering through DNA replication and subsequently templating methylation of daughter chromosome regions. However, a report in this issue (Petruk et al.) suggests that chromatin association of the methytransferase complexes themselves persists through replication and re-establishes histone methylation.

Epigenetics is the study of heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic inheritance of specific patterns of gene expression is essential for the maintenance of cell lineages. Large multiprotein complexes and posttranslational modifications of histone proteins that package DNA have been linked to both the active expression and repression of genes that define particular cell types. However, the mechanism by which this protein architecture is manipulated to allow the replication machinery to pass by but remember its original configuration for reassembly has been a topic of much study and debate. Covalent histone modifications have been implicated in epigenetic inheritance in numerous studies (Suganuma and Workman, 2011; Zhu and Reinberg, 2011), and it has been proposed that these modifications are maintained at specific genomic loci through mitotic cell divisions. A model has emerged in which modified histones on parental DNA are randomly partitioned to daughter strands during DNA replication and subsequently promote the modification of newly added histones. This mechanism would ensure that the architecture of specific loci is passed on to daughter cells (Corpet and Almouzni, 2009; Zhu and Reinberg, 2011). A provocative report now challenges, or at least provides a dramatic alternative to, this model. Petruk et al. (2012) present evidence from *Drosophila* embryos that histone modifications are actually lost during DNA synthesis and, instead, that the association of histone-modifying enzyme complexes with specific loci persists during replication and re-establishes the histone modifications after S phase.

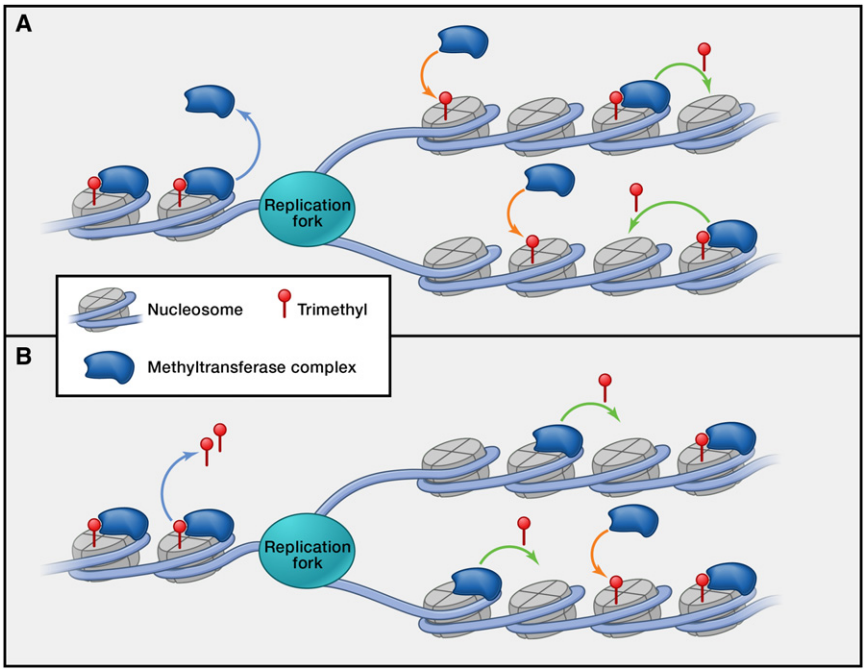

Figure 1. Inheritance of Histone Methylation Marks through DNA Replication
Alternate models for the inheritance of histone methylation marks through DNA replication are depicted. (A) Histone methylation passes the replication fork. Prior to replication, histone methyltransferase complexes (MTCs) are stably bound to nucleosomes containing trimethylated histone H3 (red dot). Upon passage of the replication fork, the MTCs are displaced from nucleosomes (blue arrow), and the methylated nucleosomes are distributed equally to the two daughter strands. New nucleosomes are assembled from the soluble pool to complement the half dose of parental methylated histones on each daughter strand (not shown). New MTCs associate with the methylated nucleosomes (orange arrows) and promote methylation of H3 on the new nucleosomes (green arrows), thereby re-establishing the methylated domain. (B) MTCs pass the replication fork. Upon passage of the replication fork, trimethylated histone H3 is lost (blue arrow), but retained MTCs are distributed on the daughter strands. The stably bound MTCs then remethylate the parental nucleosomes and methylate the new nucleosomes (green arrows), leading to recruitment of new MTCs (orange arrows) to fully re-establish the methylated domain.

Core histones, which package DNA into nucleosomes and higher orders of chromatin structure, are subject to several posttranslational modifications that can alter chromatin structure and/or serve as signals or docking sites for other proteins and enzyme complexes (Suganuma and Workman, 2011). Different types of histone modifications are often associated with different "states" of gene activity. For example, lysines (K) within histones are often acetylated at actively transcribing genes but are deacetylated at silent genes (Suganuma and Workman, 2011). Although these and other histone modifications appear to be transient, methylation of histone lysines is thought to be more stable and likely to contribute to epigenetic phenomena (Zhu and Reinberg, 2011). The function of lysine methylation differs for lysines in different positions. For example, trimethylated histone H3K4, a modification carried out by the Trithorax (Trx) methyltransferase in the Trithorax group (TrxG) complex, is associated with active genes (Mohan et al., 2011). By contrast, trimethylation of H3K27, which is carried out by the Enhancer-of-Zeste (E(z)) methyltransferase in the Polycomb group (PcG) complex, is associated with heterochromatic and other silenced genes (Simon and Kingston, 2009). Not surprisingly, the genes encoding these proteins have long been associated with epigenetic phenomena, indirectly supporting models that their modification of histones by methylation accounts for the mechanism of epigenetic inheritance during DNA replication.

At the DNA replication fork, parental histones have been found to partition randomly to the daughter strands such that each daughter has half of the parental nucleosomes. An equal amount of new nucleosomes assemble on each daughter strand from the soluble histone pool. This partitioning was thought to deliver a half dose of parental histone modifications to each daughter DNA at any given locus. This half dose of modifications was thought to reinforce the modified chromosome domain by promoting (templating) similar modification of the newly assembled nucleosomes (Figure 1A). For example, the PRC2 PcG protein complex contains the methyltransferase E(z) and EZH2, a protein that recognizes and binds H3K27m3. Thus PRC2 could bind parental nucleosomes modified by H3K27m3 via the EZH2 subunit and methylate surrounding nucleosomes on H3K27 through E(z), thereby reinforcing the modified domain (Zhu and Reinberg, 2011). Various versions of this histone modification templating and copying mechanism have been favored models for passing on a domain of modified nucleosomes to daughter chromosomes (Figure 1A).

In the current study, Mazo and colleagues use immunofluorescence and biochemical analysis to examine the presence of histone modifications during S phase in *Drosophila* embryos (Petruk et al., 2012). Surprisingly, the authors find that H3K4m3 and H3K27m3 are largely absent from cells in S phase, which were identified by the presence of the DNA replication protein PCNA. Moreover, although antibodies to histones precipitate DNA sequences also bound by PCNA, antibodies to histones modified by H3K4me3 or H3K27me3 do not. These and additional studies suggest that modified histone H3 is replaced during DNA replication with H3 lacking these modifications. Alternatively, H3 could be demethylated at these sites during replication and subsequently remethylated. In either case, these modifications appear to be lost on histone H3 during S phase and therefore unavailable to template the re-establishment of the modified domain after S phase.

How, then, can the H3K4m3 and H3K27m3 modifications be remembered and re-established after S phase? Furthering their study, Petruk et al. examined the sites of replication (replication forks) for the presence of the TrxG and PcG enzyme complexes that carry out these modifications. They find that the

TrxG protein Trx and the PcG proteins Pc and E(z) are associated with recently replicated DNA sequences also bound by PCNA. In addition, Trx, Pc, and E(z) could be found in close proximity to PCNA in a "proximity ligation assay." Thus, although the H3K4m3 and H3K27m3 marks appear to be lost in S phase, the enzyme complexes that carry out these modifications remain associated with chromatin during its replication. These data support a model in which the H3K4 and H3K27 methyl marks are lost during DNA replication but are re-established after replication by the TrxG and PcG histone methyltransferase complexes (Figure 1B).

It is not yet clear whether Trx, Pc, and E(z) remain associated with chromatin during replication or rapidly reassociate after passage of the replication fork. Interestingly, the PcG proteins Psc and Pc have been shown to stably bind chromatin during DNA replication in vitro (Francis et al., 2009). Moreover, the ability of Psc to oligomerize has lead to a proposal in which the oligomer can "bridge" the replication fork to associate with newly replicated chromatin (Lo et al., 2012). Alternatively, the ability of Trx and E(z) to bind to single-stranded DNA, as at the replication fork, could account for their retention at sites of replication (Krajewski et al., 2005). Finally, it is also possible that TrxG and PcG proteins are passed around the elongation fork by transiently interacting with replication proteins, as observed for other histone-modifying enzymes (Zhu and Reinberg, 2011). It will be important to uncover the mechanism by which these enzyme complexes pass replication forks and to extend these studies to other systems to determine whether the loss of methylated histones and retention of modifying complexes during replication are unique to rapidly dividing cells in *Drosophila* embryos or conserved among other cell types.

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LIS1 Clamps Dynein to the Microtubule

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Cytoplasmic dynein is a motor essential for numerous mechanical processes in eukaryotic cells. How its activity is regulated is largely unknown. By using a combination of approaches including single-molecule biophysics and electron microscopy, Huang et al. in this issue uncover the regulatory mechanism by which LIS1 controls the activity of cytoplasmic dynein.

Cytoplasmic dynein is a microtubule motor that carries out the majority of tasks depending on minus-end directed motility in the cytoplasm of most eukaryotic cells (Allan, 2011). Several accessory proteins modulate dynein's properties and functions. Prominent examples are the dynactin complex, LIS1 and NudE (Kardon and Vale, 2009). How these cofactors regulate dynein's cellular activities is still poorly understood. In this issue of *Cell*, Huang et al. (2012) unravel the molecular mechanism by which LIS1 regulates the motility of cytoplasmic dynein from *Saccharomyces cerevisiae*.

Cytoplasmic dynein is a fascinating enzyme. It is a large complex consisting of two dynein heavy chains and several smaller subunits. The smaller subunits associate with the N-terminal part of the heavy chains, forming the cargo binding region. The C-terminal part of the heavy chain forms the motor domain. Each motor domain consists of: (1) A hexameric AAA+ (ATPase associated with various cellular activities) ring with the major ATP hydrolysis site located in the AAA1 domain (Figure 1A); (2) The microtubule binding domain (MTBD) located at the end of an elongated antiparallel coiled coil (~15 nm) called the stalk that protrudes from AAA4 domain (Figure 1A); (3) The linker connecting AAA1 with the N-terminal sequence of the heavy chain. This linker represents the major mobile mechanical element responsible for force generation and directional movement of this motor (Cho and Vale, 2012).